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By PONG CHEH JEN

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PARTIAL FULFILMENT OF THE REQUIREMENTS FOR
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1938

REPRINTED FROM
THE JOURNAL OF BIOLOGICAL CHEMISTRY
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XXVI. THE METABOLISM OF THE BETAINE OF CYSTINE

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(Received for publication, October 20, 1938)

Little is known of the metabolic behavior of the completely methylated derivatives of the amino acids, the betaines, so widely distributed in the tissues of plants and of invertebrate animals (1, 2). The α -betaines, of most common occurrence in nature, are usually stated to be physiologically inactive and to be excreted unchanged in the urine when administered to the higher animals (1-3). Kohlrausch (3), although he accepted this conception of physiological inertness, suggested that after oral administration of the betaine of glycine to rabbits, partial demethylation occurred and trimethylamine was excreted in the urine. The conversion by partial demethylation of betaine to sarcosine as a precursor of creatine has also been suggested (4), although the experimental evidence in support of such a conversion is hardly convincing. The hypothesis that the betaines escape extensive metabolic change is based chiefly upon their isolation from the urine, a procedure fraught with difficulty and hardly quantitative.

The betaine of cystine has recently been synthesized (5) and is available for metabolic study. It is known that the sulfur of N-substituted derivatives of cystine is not oxidized to sulfate and excreted in the urine as readily as is the sulfur of cystine (6-9). Since the amino group of cystine is blocked by methyl groups in the cystine betaine, if cystine betaine were not demethylated, it would be anticipated that after its administration, the sulfur would escape oxidation, and that the sulfur of cystine betaine would constitute a part of the organic sulfur of the urine. If, on the other hand, any extensive demethylation occurred, as suggested by Kohlrausch, the sulfur of the demethylated derivative might be

expected to be oxidized and to appear in the urine in the sulfate sulfur fraction.

If partial demethylation of cystine betaine occurred biologically, a reaction analogous to the suggested conversion of the betaine of glycine to creatine, the formation of N,N'-dimethylcystine should result. The biological behavior of this cystine derivative has not been extensively investigated, since its synthesis has presented difficulties (10). If the theory that N-monomethylamino acids are converted to the corresponding α -keto or α -hydroxy acids is correct (11, 12), N,N'-dimethylcystine should be readily oxidized and utilized. The N-methyl derivatives of homocystine and methionine have been shown to promote growth of the young white rat, when added to a diet known to be deficient in its content of total sulfur-containing amino acids and low in cystine (13). Amino-N-methyltryptophane (14) and amino-N-methylhistidine (15) can be utilized for growth by the young white rat, replacing tryptophane and histidine respectively.¹

In view of these considerations, a study of the metabolic behavior of cystine betaine has been undertaken. The presence of sulfur in the molecule has made possible a method of approach to the study of the behavior of the betaines, other than that of the isolation of the compound under investigation from the urine. The distribution of urinary sulfur after oral and parenteral administration of cystine betaine to the rabbit has been studied. In view of the results obtained with N-methyl derivatives of homocystine and methionine (13), the utilization of cystine betaine for the promotion of growth of the young white rat was investigated. No indications that the betaine of cystine undergoes significant metabolic changes in the organism of these two species were obtained.

Oxidation of Sulfur of the Betaine of Cystine—The general pro-

¹ It is, of course, not permissible to assume, without direct experimental proof, that the α -hydroxy or α -keto derivatives of the naturally occurring amino acids all follow similar metabolic paths. These derivatives of cystine are difficult to prepare in a state sufficiently pure for convincing metabolic study. Similarly, not all the amino-N-methyl derivatives of the amino acids show the same biological behavior. In contrast to the α -N-methyl derivatives of tryptophane, histidine, and methionine, α -monomethyllysine failed to replace lysine for purposes of growth in the organism of the white rat (16).

cedure and analytical methods were those commonly employed in this laboratory in similar studies of the oxidation of sulfur compounds (17). Adult male rabbits weighing approximately 3 kilos served as experimental animals. In order to afford a basis for comparison between the metabolic behavior of cystine and its betaine, the oxidation of the sulfur of cystine was also studied in control experiments with each animal.

The cystine was prepared from hair, and the betaine of cystine according to the procedure of Schubert (5). The specific rotation of the *l*-cystine (1 per cent solution in *N* hydrochloric acid) measured at 25° and with the use of the sodium flame was -210°. Both the cystine and the betaine of cystine were shown to be of satisfactory purity by analyses for nitrogen and sulfur.

The readily soluble betaine was administered in aqueous solution, while the less soluble cystine was converted to the sodium salt by dissolving in the theoretical amount of a solution of sodium carbonate. Both compounds were fed or injected in amounts equivalent to 0.333 gm. of sulfur.

The results are summarized in Table I. The extra sulfur as here presented was obtained by subtracting the average daily urinary sulfur excretions of the control fore and after periods from the sulfur excretions of the various experimental periods. The extra sulfur was also calculated by subtracting the "predicted" sulfur values from the amounts excreted on the days the various experimental substances were administered. The average N:S and N:SO₄S ratios of the urines of the control days were determined. On the basis of these ratios, the predicted sulfur excretions were obtained by dividing the nitrogen values of the experimental days by the appropriate ratio. In this method of calculation, any extra sulfur which might result from a general increase of protein metabolism associated with an increased nitrogen excretion is taken into consideration. In previous studies of sulfur metabolism, we have occasionally observed a stimulation of tissue catabolism and an associated increase in the elimination of both nitrogen and sulfur. No such increased catabolism was observed in the present series and the extra sulfur values calculated by the two methods were in good agreement. For example, the extra sulfur values of Experiment E-1, calculated from the predicted sulfur values, were 0.22 and 0.157 gm. (total and sulfate sulfur re-

spectively), which may be compared with the corresponding values of 0.226 and 0.150 gm. calculated from the average normal urinary sulfur excretion. Similar values for Experiment F-7 were 0.300 and 0.018 gm. respectively, as compared with 0.313 and 0.030 gm.

The data require little comment. After parenteral administration, the sulfur of the betaine of cystine was not oxidized and excreted in the urine as sulfate sulfur, since the extra sulfur of the urine consisted in large part of organic sulfur. Thus, only 5 and

TABLE I

*Distribution of Extra Sulfur in Urine Excreted in 24 Hour Period
Immediately Following Administration of l-Cystine and the
Betaine of l-Cystine*

Extra sulfur values are expressed as per cent of the total sulfur administered, the figures in parentheses showing the percentage distribution of the extra sulfur between sulfate and organic sulfur. The sulfur content of the compounds fed was, in all cases, 0.333 gm. The letters of the experiment number are used to designate the individual experimental animals, while the numeral refers to the number of the experiment. All experiments bearing the same letter were carried out with the same animal.

Experiment No.	Mode of administration	Compounds fed	Extra sulfur excreted		
			Total	Sulfate	Organic
E-1	Oral	<i>l</i> -Cystine	67.6	47.7 (71)	19.9 (29)
F-5	"	"	74	55 (74)	19 (26)
E-4	Subcutaneous	"	76	52 (69)	24 (31)
F-8	"	"	93.4	66 (72)	27.4 (28)
E-2	Oral	Cystine betaine	61.5	19.2 (31)	42.3 (69)
F-6	"	" "	82.5	27 (33)	55.5 (67)
E-3	Subcutaneous	" "	82	4.5 (5)	77.4 (95)
F-7	"	" "	90	5.4 (6)	84.6 (94)

6 per cent of the extra sulfur were excreted as sulfate sulfur after subcutaneous injection of the betaine, percentages which may be contrasted with 69 and 72 per cent of the extra sulfur excreted as urinary sulfate sulfur when cystine was injected similarly into the same animals. These results are comparable to those previously obtained by one of us (L.) in studies with α -phenylureidocystine (6) and dibenzoylcystine (8) and indicate that blocking the amino group by complete methylation results in a failure of the oxidation of the sulfur of the cystine derivative to sulfate sulfur.

In experiments in which the betaine was fed, there was observed some increase in the excretion of sulfate sulfur, an increase which is believed to be greater than the error of the experimental procedure and to represent oxidation of part of the betaine sulfur. We have discussed previously the possible modification of the metabolic behavior of cystine and related sulfur derivatives (18) by the microflora of the intestine. This effect of the microflora has recently been the subject of direct experimental study (19). We have observed similar slight increases in the excretion of sulfate sulfur after oral administration of substituted derivatives of

TABLE II

Food Consumption and Increase in Weight of Rats Fed Basal (Cystine-Deficient) Diets Supplemented by Cystine and Betaine of Cystine (Litter 3)

The animals of Group VI were females; the other animals, males. The experimental feeding period was of 42 days duration.

Group No.	Rat No.	Supplement to basal diet	Food average daily	Weight initial	Gain	
					Average daily	Per 100 gm. food
			gm.	gm.	gm.	gm.
IV	3214	Cystine	4.8	63	1.38	29.0
	3212	Betaine of cystine	4.8	63	0.37	7.6
V	3225	Cystine	5.2	67	1.37	21.0
	3226	"	5.2	67	1.22	23.8
VI	3327	Betaine of cystine	5.3	69	0.27	5.2
	3237	Cystine	5.2	62	1.19	23.8
	3333	Betaine of cystine	5.2	69	0.21	3.9
	3338	" " "	5.2	63	0.35	6.9

cystine (6, 8). The greater part of the extra sulfur in the present series, as in the previous experiments, is to be found in the organic sulfur fraction of the urine.

Availability of Betaine of Cystine for Growth of Young White Rats—Young rats in litter units were fed a basal diet low in its content of cystine for a period of 14 days. The diet of White (20) was modified to contain 5 per cent instead of 6 per cent of casein (Labco brand, vitamin-free) and 1 per cent of agar. Water-soluble and fat-soluble vitamins were supplied by the separate administration of dried brewers' yeast (approximately 400 mg.)

and cod liver oil (3 drops) daily. During this preliminary period, the animals either failed to gain or showed slight increases in weight. After the preliminary feeding period, the animals were divided into two groups and the paired feeding method employed. One group received cystine as a supplement (0.5 per cent); a second group received the betaine of cystine in amounts equivalent in sulfur content to 0.5 per cent of cystine. With one litter, a third group was continued on the basal diet also. Experiments with three litters, which included twelve rats on the diet containing the betaine and ten rats on the diet containing the cystine supplement, were conducted over a period of 42 days. The results were uniform and may be illustrated by the detailed presentation of the data for a single litter (Table II). The animals which received supplementary cystine showed good growth responses, while their litter mates which received the betaine of cystine as a supplement in place of cystine failed to grow satisfactorily, the rate of growth being similar to that of animals to whose diet (basal) no supplement was added. It is evident that the betaine of cystine is not adequate for the promotion of growth when used as a supplement to a diet known to be low in its content of cystine. These results are comparable to those of Jackson (21) who supplemented a diet deficient in its content of tryptophane with the betaine of this essential amino acid.

SUMMARY

1. After the subcutaneous injection of the betaine of *l*-cystine into rabbits, a study of the distribution of the extra sulfur of urine did not indicate any oxidation of the sulfur of this cystine derivative, since the extra sulfur observed was present in the organic sulfur fraction. The slight increase in oxidized (sulfate) sulfur of the urine observed after oral administration of cystine betaine is believed to be the result of the activity of the microflora of the intestine, which yields products capable of being oxidized to sulfate.

2. Cystine betaine did not function as did cystine in the promotion of growth of young white rats fed a basal diet low in its content of cystine.

3. These results are consistent with the generally accepted belief that the completely methylated derivatives of the α -amino

acids, the α -betaines, are not readily broken down and utilized in the metabolism of the higher animals.

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